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The Dominant Mutation *Glazed* Is a Gain-of-Function Allele of *wingless* That, Similar to Loss of APC, Interferes with Normal Eye Development

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Dominant mutations have served as invaluable tools for *Drosophila* geneticists. Here we analyze the dominant eye mutation *Glazed* (*Gla*) that was described by T. H. Morgan more than 50 years ago. We show that *Gla* causes the loss of photoreceptor cells during pupal stages, in a process reminiscent of apoptosis, with a concomitant overproduction of eye pigment. This phenotype is very similar to that caused by the loss of D-APC, a negative regulator of Wingless (Wg) signal transduction. Genetic analyses reveal however that the *Gla* gain-of-function phenotype can be reverted to wild-type. By generating a P-element-induced revertant of *Gla* we demonstrate that *Gla* is allelic to *wg*. The molecular lesion in *Gla* indicates that the insertion of a *roo* retrotransposon leads to ectopic expression of *wg* during pupal stages. We show that the *Gla* phenotype is similar to that caused by ectopic expression of Wg driven by the *sevenless* (*sev*) enhancer. In both cases Wg exerts its effect, at least in part, by negatively regulating the expression of the Pax2 homolog *sparkling* (*spa*). *Gla* represents not only the first dominant allele of *wg*, but it may also be the first allele ever described for *wg*.

INTRODUCTION

The compound eye of *Drosophila melanogaster* serves as a paradigm to study and understand how complex patterns arise from undifferentiated cells. Over the past years, significant insight into some of the mechanisms that control recruitment and patterning of retinal cells has been gained (Wolff and Ready, 1993; Dickson and Hafen, 1993). As entry points to decipher the cellular communication processes governing pattern formation, mutations can be used in which the regular pattern of ommatidial units is disturbed.

Here we have investigated the molecular nature and developmental consequences of the dominant mutation *Glazed* (*Gla*) (Morgan *et al.*, 1936). *Gla* is frequently used to follow the inheritance of chromosome 2. *Gla* mutant adults have narrowed eyes that are reduced in size. The facets of these eyes coalesce into a gleaming, smooth sheet (Lindsley and Zimm, 1992).

We show that *Gla* is a unique allele of the *wingless* (*wg*) gene, which causes ectopic expression of *wg* during eye development. Our molecular analysis indicates that the *Gla* mutation is not caused by a chromosomal rearrangement, but rather by the integration of a transposable element into the promoter region of the *wg* gene. We demonstrate that the *Gla* phenotype can be reverted by preventing *wg* transcription from the *Gla* allele. Furthermore, we find that ectopic expression of *wg* under the control of the *sevenless* (*sev*) enhancer partially mimics the *Gla* phenotype. Common consequences of both genotypes are the loss of photoreceptor cells by apoptosis and a concomitant overproduction of eye pigment, phenotypes that are also caused by inactivation of the *Drosophila* homolog of the tumor suppressor APC (Ahmed *et al.*, 1998).

MATERIALS AND METHODS

Drosophila Strains

Most *Bc,Gla* chromosomes that are used in the laboratory carry an additional dominant mutation *Ellipse* (*Elp*) which itself interferes with normal eye development enhancing the *Gla* phenotype. Recombinants which have lost the *Elp* mutation were generated by exchanging the distal portion of the second chromosome with the corresponding part of a bw^- chromosome. The *wg* null allele ($wg\text{-}lacZ^{ro216}$) was kindly provided by E. Wieschaus.

[1] These authors contributed equally to this work.

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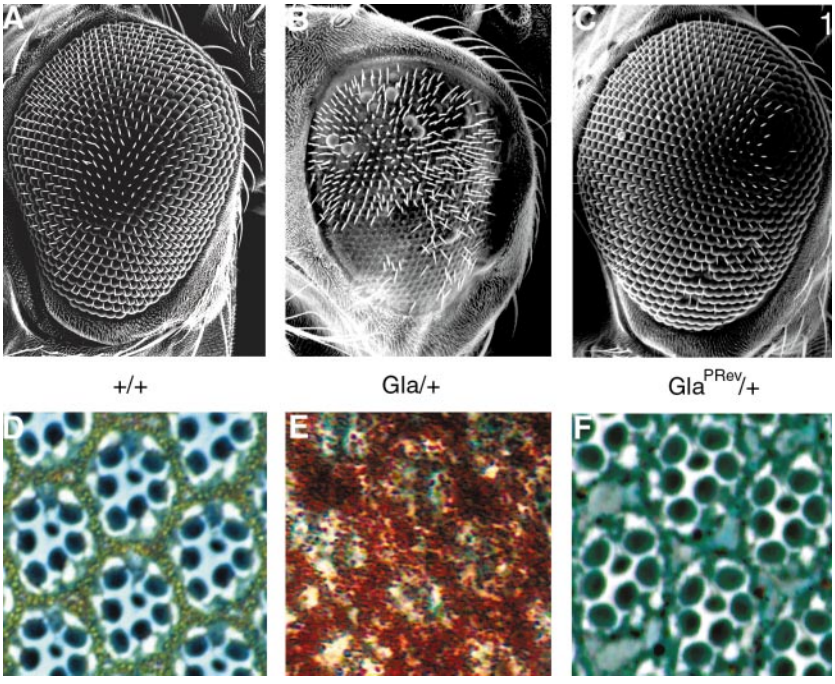

FIG. 1. The adult *Gla* mutant phenotype. Scanning electron micrographs of adult eyes of wild-type (A), *Gla* (B), and *Gla*^{PRev} (C) flies. Note the almost complete absence of photoreceptor cells and the excess of pigment granules in tangential eye sections of *Gla* mutants (E) compared to wild type (D). The regular array of ommatidia is restored in *Gla*^{PRev} flies (F). The *Elp* mutation which enhances the *Gla* eye phenotype in most laboratory strains has been removed by recombination (see Materials and Methods).

Confocal and SEM Microscopy

Adult flies were stored in 70% acetone before they were critical-point-dried and gold-coated to be examined in a Hitachi 4000 scanning electron microscope. Confocal images were obtained with a Sarrastro 2001 inverted confocal microscope system (Molecular Dynamics).

Transgenes

Construction of pEB-control. An approximately 3.5-kb *Pst*I–*Bam*HI fragment (*hsp27* basal promoter and *lacZ* gene) was removed from pßn27.1 (Riddihough and Ish-Horowicz, 1991) and cloned into *Pst*I, *Bam*HI-digested CaSpeR4.

Construction of pEB-Gla. PCR was performed on pwg-6 (Baker, 1987) using primers 5′-GCACGCAGCTGCAATGCAGG-3′ (Pr1, Fig. 3A) and 5′-GCTCTAGA-GGGTTTATCTGTTCGACG-3′ (Pr2, Fig. 3A). The 0.6-kb fragment was digested with *Pvu*I (partial digest since there are two *Pvu*I sites in the PCR product; see also Fig. 3A) and with *Xba*I. The smaller fragment and a 1.5-kb *Pst*I–*Pvu*I fragment of the rescued plasmid p766 were cloned together into *Pst*I, *Xba*I-digested Bluescript (SK$^-$), generating p844-Gla. p844-Gla was digested with *Eco*RI, treated with T4 DNA polymerase, and digested further with *Xba*I. The blunt end to *Xba*I fragment was cloned into pEB-control digested with *Not*I (T4 DNA polymerase treated) and *Xba*I. This resulted in the final construct pEB-Gla.

Construction of sev-wg. The *sev-wg* transgene carries two copies of the *sev* enhancer and the *hsp70* promoter in front of the *wg* coding region. The construct was made by replacing the *sev*-specific coding sequences in sev-S11 (Basler *et al.*, 1991) with those of *wg* (Struhl and Basler, 1993). To avoid the toxicity of injecting an *hsp70-wg* construct, a flp-out cassette (Struhl and Basler, 1993) was inserted between the *hsp70* promoter and the *wg* coding sequence. This cassette was subsequently excised in the germ line by exposing transformants transiently to *flp* recombinase.

Plasmid Rescue from Gla^{PRev} Genomic DNA (p766, p768)

The P-lacW P element (Bier *et al.*, 1989) was used for the reversion of the *Gla* mutant phenotype. Plasmid rescue was performed according to the protocol of Bier *et al.* (1989). *Gla*^{PRev} genomic DNA was digested either with *Bam*HI or with *Eco*RI obtaining a 7-kb or an 11-kb fragment, respectively. The sequence data submitted to GenBank (Accession No. AF100158) was not confirmed by sequencing both entire strands but was found to be identical to parts of the *wg* 5′ sequence, GenBank Accession No. U84292.

Staining Procedures

Cobalt sulfide stainings were carried out as described in Wolff and Ready (1991).

Antibody stainings. Late third instar larvae were dissected or transferred to a fresh tube and aged at 25°C for 24–60 h. Eye discs were recovered and stained for β-galactosidase activity or Elav,

Spectrin, or Wg, respectively, by standard immunofluorescence procedures (as described in Basler and Struhl, 1994). A rabbit anti-Wg antibody (kindly provided by M. van den Heuvel) was used to detect the Wg protein. The rat anti-Elav monoclonal antibody was a gift of G. Rubin. A polyclonal antibody described in Pesacreta *et al.* (1989) was used to detect the Spectrin protein. Immunostaining of pupal eye discs with anti-Spa antibody was carried out as described in Fu and Noll (1997).

RESULTS

The Gla Mutant Phenotype

The X-ray-induced dominant *Gla* mutation was first described by Morgan, Bridges, and Schultz in 1936. It was named after the glazed appearance of the strongly pigmented eyes of animals carrying the mutation. Based on the association of *Gla* with the inversion *In(2LR)Gla* [=*In(2LR)27D;51E*], the mutation was believed to map to one of its two chromosomal breakpoints [cytological positions refer to the revised map of Bridges (1942)]. *Gla* serves as a dominant genetic marker on the *Bc,Gla* balancer chromosome where *In(2LR)Gla* has been superimposed on an additional inversion [*In(2L)t*, Woodruff and Ashburner, 1979]. The *Bc,Gla* chromosome that is used most frequently carries another dominant mutation, *Ellipse* (*Elp*), which itself interferes with normal eye development, thus enhancing the *Gla* phenotype. For our studies we generated a *Bc,Gla* chromosome from which the *Elp* mutation has been removed by recombination (see also Materials and Methods).

A study of the *Gla* mutation was initiated because it causes a severe disruption of eye morphology indicating that some fundamental processes of eye development are affected. Figure 1B shows a scanning electron micrograph of a *Gla* mutant eye. Only occasionally normal-looking ommatidial units can be seen in dorsal regions. However, a regular pattern of structures reminiscent of the normal hexagonal array of ommatidia is covering the eye surface. In most of the preparations ommatidial bristles are missing in the anterior-ventral part where the *Gla* mutant phenotype is generally more pronounced. Tangential eye sections reveal that almost no photoreceptor cell structures are present in *Gla* mutants (Fig. 1E). Instead, most of the eye appears to consist of pigment cells since pigment granules are highly abundant over the entire section. The overall distribution of these granules forms a faint regular pattern reminiscent of the arrangement of ommatidia in wild-type eye sections (Fig. 1D).

The presence of regular structures on the surface, as well as in tangential sections of adult eyes, suggests that the initial steps of eye patterning and differentiation might not be affected in *Gla* mutants. To test this assumption we stained late third instar larval eye discs with the anti-Elav antibody which serves as an early neuron-specific nuclear marker (Campos *et al.*, 1987). In wild-type discs, the regularly spaced clusters formed by the nuclei of the developing photoreceptor cells are visible in a characteristic pattern behind the morphogenetic furrow (for review see Wolff and Ready, 1993). The Elav staining pattern of *Gla* mutant discs is indistinguishable from that of wild-type discs of the same developmental stage indicating that all photoreceptor cells initiate normal differentiation (data not shown).

To investigate the time point at which photoreceptor cells are lost, we monitored Elav expression in pupal eye discs at different stages. As an internal control we made use of a P-element insertion into the *Gla* locus (Gla^{PRev}) that reverts the dominant *Gla* phenotype (see below, Fig. 1C). By mobilizing the P element by means of the P transposase, the dominant *Gla* phenotype can be restored. Animals carrying both the Gla^{PRev} P element and a transgene coding for the P transposase possess mosaic eyes composed of wild-type and *Gla* mutant patches. In such dysgenic eye discs, the Elav staining pattern develops and persists as in wild-type discs up to about 30 h of pupal development. It is only between 30 and 40 h of pupal development that mutant and wild-type patches differ. At this stage, Elav staining completely disappears in the *Gla* mutant patches, but not in the surrounding wild-type tissue. Figure 2A shows a 30- to 40-h pupal disc containing clones of *Gla* cells in an otherwise Gla^{PRev} background. While in the wild-type (Gla^{PRev}) tissue the normal circular arrangement of Elav-positive photoreceptor cell nuclei can be observed, the nuclei are disintegrating in the mutant patches, as indicated by the condensed appearance of the Elav staining (Fig. 2A). This same observation has previously been taken as an indication of apoptosis in the developing eye (Hay *et al.*, 1994). In pupal discs older than 40 h, no more Elav-positive photoreceptor cells are found in mutant clones (Fig. 2B). Notably, however, the evenly distributed nuclei of the bristle neurons remain Elav positive in the wild-type as well as in the mutant tissue during the period when the photoreceptor cells disappear (Fig. 2B). *Gla* mutants therefore lack photoreceptor cells because these cells die between 30 and 40 h of pupal development.

To investigate the consequences of photoreceptor cell loss on the remaining cell types, 48- to 60-h pupal eye discs were stained with an antibody that recognizes Spectrin, a ubiquitous component of cell membranes. In wild-type eye discs, a flower-like pattern of secondary and tertiary pigment cells surrounds every ommatidial unit (Fig. 2D). Whereas the slim, ellipsoidal secondary pigment cells are shared between two ommatidial units, the bigger, triangular tertiary pigment cells are shared between three ommatidial units (reviewed in Wolff and Ready, 1993). In *Gla* mutant eye discs, an essentially normal number of pigment cells is found. However, their shape is predominantly rectangular suggesting that most of the pigment cells have adopted a tertiary rather than a secondary pigment cell fate (Fig. 2E). Due to the lack of specific markers we could not further investigate the identity of these pigment cells. The massive excess of pigment granules in adult *Gla* eyes suggests that these cells then may undergo either hypertrophy or additional divisions, or both.

Gla Is a Gain-of-Function Allele of wingless

To test whether the *Gla* phenotype is caused by a gain-of-function mutation, we sought to revert the phenotype by X-ray-induced inactivation of the *Gla* gene. Five X-ray-induced revertants with wild-type eyes were recovered from a total of 10,000 X-ray-treated *Bc,Gla* chromosomes, showing that *Gla* is in fact a gain-of-function mutation.

For further characterization and cloning of *Gla*, precise mapping of the locus was required. Since *Gla* is located on a balancer chromosome carrying two inversions, meiotic mapping of the mutation was not possible. For the same reason, potential newly induced chromosomal breakpoints could not be mapped cytologically on the polytene chromosomes of the X-ray revertants. We therefore carried out an additional screen with the aim of isolating P-element-induced revertants of *Gla*. From 10,000 flies screened, a single P-element insertion was recovered, Gla^{PRev}, which almost completely reverts the *Gla* mutant phenotype to wild type (Figs. 1C and 1F).

Since the P element used also carries a *lacZ* reporter gene to uncover enhancer elements located in the vicinity of its insertion site (see Materials and Methods), we stained third instar eye antennal, leg, and wing discs for *lacZ* expression. In all imaginal discs, Gla^{PRev} expression was found in distinct patterns indistinguishable from those of the *wingless* (*wg*) gene (Fig. 3D; Baker, 1988). This raised the possibility that the P element in Gla^{PRev} had inserted in close proximity of the *wg* locus. To precisely map the Gla^{PRev} insertion site, genomic DNA sequences on both sides of the P-element insertion were cloned by plasmid rescue, generating plasmids p766 and p768 (Fig. 4A, see Materials and Methods). These plasmids where then used as probes for *in situ* hybridization to wild-type polytene chromosomes. With p768, a single signal at cytological position 28A1-3 was detected (data not shown). This corresponds to the map position of the *wg* gene, providing further evidence that the P element in Gla^{PRev} had inserted near the *wg* locus. When p766 was used as a probe, approximately 100 independent signals distributed over all four chromosomes were detected, only 1 of which corresponded to the *wg* locus. None of the signals corresponded to the chromosomal breakpoints reported for the *Bc,Gla* chromosome (Woodruff and Ashburner, 1979; Lindsley and Zimm 1992), indicating that the breakpoints are unlikely to contribute to the *Gla* mutation.

To test whether Gla^{PRev} is interfering with endogenous *wg* function, we analyzed homozygous mutant embryos. Since both the original *Bc,Gla* and the Gla^{PRev} chromosomes already cause lethality when homozygous (most probably due to the inversion breakpoints), we crossed the Gla^{PRev} and the *Bc,Gla* chromosomes to a *wg* null allele ($wg^{lacZro216}$). Whereas $Bc,Gla/wg^{lacZro216}$ animals developed into viable adult flies, $Gla^{PRev}/wg^{lacZro216}$ animals died, exhibiting a strong segment polarity phenotype indistinguishable from that of homozygous *wg* null mutants (Figs. 3A–3C). Because the *Gla* mutant phenotype can be reverted by a single P-element insertion that abolishes *wg* function, we conclude that *Gla* is a gain-of-function allele of *wg*.

The Gla Mutation Is Caused by a roo Element Inserted into the wg Locus

To investigate the molecular nature of the *Gla* mutation, p766 and p768 were probed by Southern blot analysis to various genomic clones spanning the *wg* locus (Baker, 1987; see also Materials and Methods, Fig. 4A). Only clone pwg-6 hybridized to both probes simultaneously, indicating that in Gla^{PRev} the P element had inserted 5′ to the *wg* coding region. All other genomic clones hybridized to either p766 or p768 only, revealing the orientation of the P element with respect to the *wg* gene and indicating that the p768 rescue fragment contained part of the *wg* coding region (Fig. 4A). Partial sequencing of p768 demonstrated that the P element had inserted 370 bp upstream of the *wg* translation initiation site (Rijsewijk *et al.*, 1987). This result indicated that the molecular cause for the *Gla* mutation is likely to map further upstream (with respect to the *wg* coding region) of the P-element insertion site, possibly in the region encompassed by p766. Restriction map comparison of the p766 rescue fragment and the wild-type genomic clone pwg-6 indeed uncovered a rearrangement present in the p766 sequence (data not shown, see below).

As with the intact p766 rescue fragment, subfragments 1 to 5 of p766 (Fig. 4A) produced multiple signals when used as hybridization probes to wild-type polytene chromosomes, suggesting that the breakpoint between the "foreign" DNA sequence and the genomic *wg* sequence may be included within the terminal 1.8-kb subfragment 5 of p766 (data not shown). The genomic nucleotide sequence upstream of the *wg* coding region as well as the sequence of subfragment 5 was determined (GenBank Accession No. AF100158). Sequence comparison revealed that most of subfragment 5 was identical to *wg* genomic sequences, except for a 0.5-kb stretch near the *Pst*I site. A database search revealed that 429 bp of this stretch are identical to the long terminal repeat (LTR) of the transposable element *roo* (Scherrer *et al.*, 1982). Further restriction map comparison of the p766 rescue fragment with *roo* element sequences confirmed that in *Gla* mutants a *roo* element has inserted 1.5 kb upstream of the *wg* coding region (Fig. 4A, GenBank Accession No. AF100158). *roo* transposable elements have a length of 8.7 kb and are flanked by 429-nucleotide LTRs (Scherrer *et al.*, 1981, 1982; Meyerowitz and Hogness, 1982).

As described above, the *Gla* mutation is viable over a *wg* null mutation. Thus, none of the *cis*-acting elements of the *wg* 5′ region responsible for *wg* expression appears to be affected by the *roo* element insertion. However, in Gla^{PRev}, regulatory elements, the transcription unit, or the interactions among them must be severely impaired, as this allele behaves as a *wg* null.

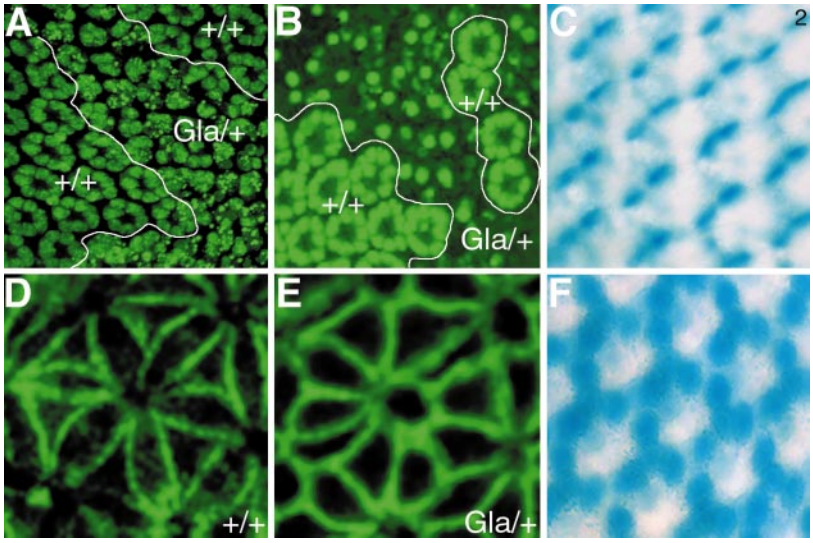

FIG. 2. The pupal *Gla* mutant phenotype. (A, B) Anti-Elav staining of pupal eye imaginal discs of dysgenic Gla^{PRev} flies 30–40 h APF (A) and >40 h APF (B). The nuclei of photoreceptor cells of *Gla* mutant clones start to disintegrate 30–40 h APF. At time points later than 40 h only few Elav-positive nuclei are found in mutant tissue which are likely corresponding to the bristle precursor cells. (D, E) Anti-Spectrin staining of the basal membrane of wild-type (D) and *Gla* mutant (E) pupal eye discs. Note the disarrangement and abnormal shape of pigment and bristle precursor cells in *Gla*. The identity of the mutant cells can no longer be deduced by morphology. (C, F) *LacZ* expression in pupal eye discs of pEB-*Gla* transformants, 24 h (C) and 40–60 h (F) APF. The expression of the *lacZ* reporter gene starts to appear in a striped pattern 24 h APF in the primary pigment cells, eventually leading to expression in all bristle precursor nuclei surrounding the photoreceptor cells >48–60 h APF (for details, see text and Materials and Methods).

The LTR of the roo Element Is Responsible for Ectopic wg Expression in the Pupal Eye Imaginal Disc

More than 20 distinct families of retrotransposons containing LTRs have been identified in *D. melanogaster*. Extensive analysis of retrotransposon transcription in cultured cells as well as *in vivo* studies revealed that the LTRs of all investigated family members bear *cis*-acting elements, controlling their specific spatial and temporal expression (Arkhipova and Ilyin, 1992; Brookman *et al.*, 1992; Ding and Lipshitz, 1994). Thus, an integration of a retrotransposon in the vicinity of a particular host gene may impose novel patterns of expression on that gene (Ding and Lipshitz, 1994; Mozer and Benzer, 1994). We wanted to analyze whether the LTRs of the *roo* transposable element might be responsible for driving ectopic *wg* expression in the developing eyes of *Gla* mutants, thereby causing the specific eye phenotype. However, as described above, the *lacZ* reporter gene in Gla^{Prev} animals is expressed in a wild-type *wg* pattern. No ectopic β-galactosidase activity was observed, neither in late third instar larval nor in staged pupal eye imaginal discs [12–48 h after puparium formation (APF)]. Similarly, antibody stainings for Wg protein in *Gla* mutant larval and pupal eye discs revealed no ectopic *wg* expression, suggesting that the *Gla* mutant phenotype might be caused by levels of ectopic Wg that are below our limits of detection.

In an attempt to uncover the time point and pattern of *wg* misexpression in *Gla* mutants, a *Gla*-specific 2.2-kb DNA fragment located upstream of the *wg* coding region, and including both a *roo* LTR and the *wg* promoter, was fused to a *lacZ* reporter gene, generating plasmid pEB-Gla (see Materials and Methods, Fig. 4B). In pEB-Gla the translation initiation site of *wg* was replaced by that of the *lacZ* gene. As a control the *lacZ* gene was also fused to the minimal *hsp27* promoter (pEB-control) to monitor a possible influence of P-element vector-specific enhancer sequences on β-galactosidase expression.

Seven independent transformant lines were tested by X-Gal staining of pupal eye discs from various stages (12–48 h), as well as of third instar larval eye discs. Five lines showed β-galactosidase expression in secondary and tertiary pigment cells as well as in bristle precursor cells starting at 24 to 48 h after puparium formation (Fig. 4A). No β-galactosidase activity was detectable in eye discs of 10 independent lines which carry the pEB-control vector (data not shown). This strongly suggests that in *Gla* mutants, *roo*-LTR-driven *wg* expression in the differentiating pigment and bristle cells is responsible for the loss of photoreceptor cells.

Ectopic wg Expression under the Control of the sevenless Enhancer Partially Phenocopies the Gla Mutant Phenotype

To confirm that ectopic *wg* expression can interfere with differentiation of ommatidial cells, we expressed the *wg* cDNA under the control of the *sevenless* (*sev*) enhancer (*sev-wg*, see Materials and Methods and Brunner *et al.*,

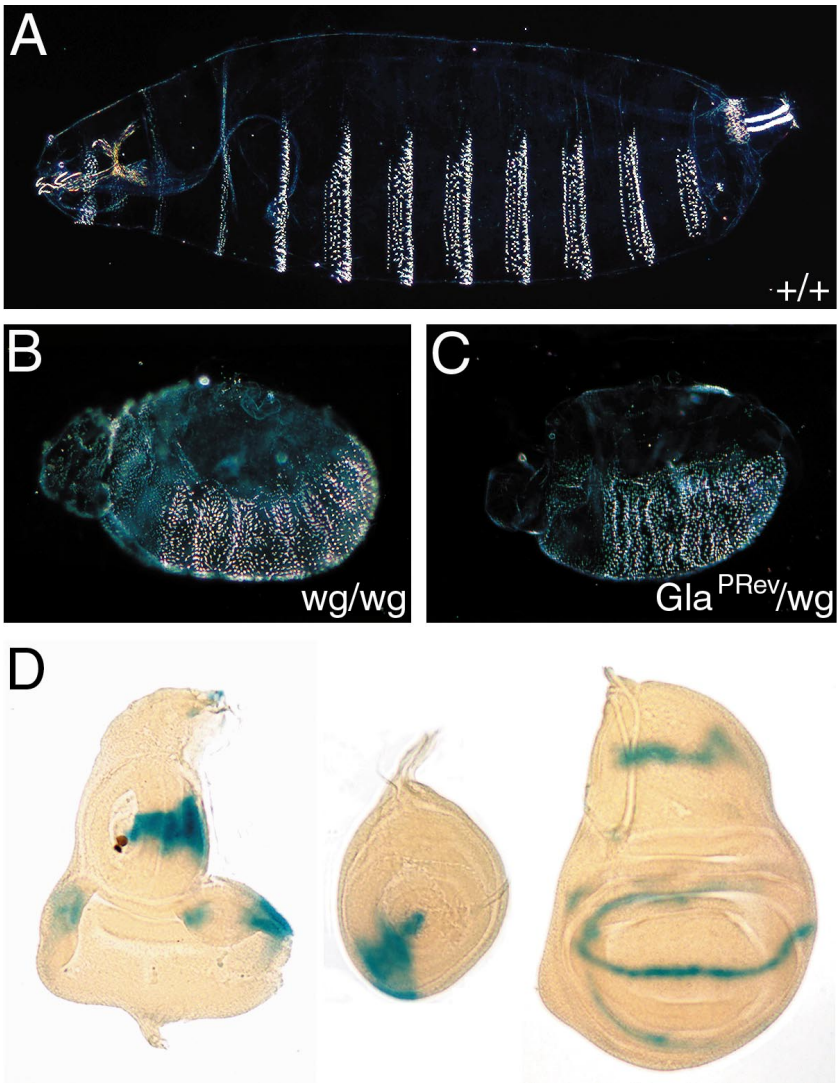

FIG. 3. Gla^{PRev} is a *wg* loss-of-function allele. Cuticle preparations of a wild-type (A), a $wg^{lacZro216}/wg^{lacZro216}$ (B), and a $Gla^{PRev}/wg^{lacZro216}$ mutant embryo (C) 24 h after oviposition. The embryo in C is indistinguishable from that of *wg* zygotic null mutants (B). D shows X-Gal stainings of third instar imaginal discs (eye antennal, leg, and wing disc from left to right) of $Gla^{PRev}/+$ third instar larvae. Ectopic expression in the eye imaginal disc could not be detected.

1997; see also Cadigan and Nusse, 1996). In third instar larval eye discs the *sev* enhancer drives gene expression posterior to the morphogenetic furrow, where *wg* is normally not expressed, in a subset of photoreceptor and cone cell precursors (Tomlinson *et al.,* 1987; Basler *et al.,* 1989). The time point of ectopic *wg* expression in *sev-wg* eye discs is therefore 24 to 48 h earlier than in *Gla* mutants and occurs in a different subset of cells.

Transformants carrying a single copy of the *sev-wg* transgene have smaller and narrower eyes than wild-type individuals (Fig. 5A). Moreover, the regular hexagonal array of the compound eye is disturbed, and all interommatidial bristles are lost. Ommatidia are also missing, predominantly in anterior-dorsal regions. In tangential sections through adult eyes, misoriented ommatidia or ommatidia with missing photoreceptor cells are found. As in *Gla* mutants an excess of pigment granules is present (Fig. 5B). Cobalt sulfide and anti-Spectrin stainings showed that up to four additional secondary pigment cells form per ommatidium (Fig. 5C). Therefore, the increased pigmentation in *sev-wg* transformants is probably due to the presence of additional pigment cells.

To investigate whether the phenotypes described here are dosage sensitive, we also expressed *wg* under the control of the *sev* enhancer using the yeast Gal4/UAS transactivation system (Brand and Perrimon, 1993). The Gal4/UAS system is temperature sensitive with lower temperatures resulting in lower levels of target gene expression (Wilder and Perri-

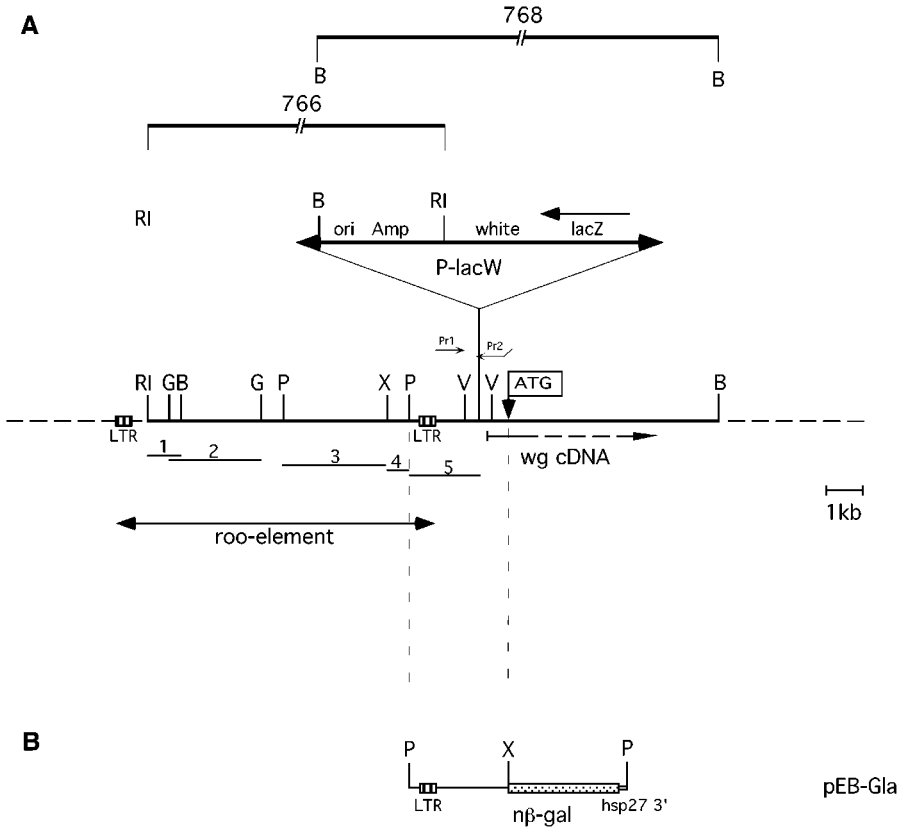

FIG. 4. (A) Map of the *wg* locus of Gla^{PRev} mutants. The P-lacW element is inserted 42 bp upstream of the *wg* cDNA2 described by Rijsewijk *et al.* (1987). Plasmid rescue clones p766 and p768 were generated by an *Eco*RI (RI) digest and a *Bam*HI (B) digest, respectively (for a detailed description, see Materials and Methods). Not all restriction sites indicated [*Bam*HI (B), *Bgl*I (G), *Pst*I (P), *Xba*I (X), *Pvu*I (V)] are unique in the region depicted. Probes 1 to 5 were used for *in situ* hybridization to polytene chromosomes (see text). The *Pvu*I and the *Pst*I sites (ATG) as well as the primers (Pr1 and Pr2) used for the construction of pEB-Gla are indicated (see Materials and Methods). (B) Structure of the pEB-Gla reporter gene. The 2.2-kb upstream region, including the LTR of the "left" arm of the *roo* element (Lindsley and Zimm, 1992), was fused to a *lacZ* reporter gene such that the AUG of the *wg* cDNA was replaced by the AUG of the reporter gene (for a detailed description, see Materials and Methods).

mon, 1995). We found that animals raised at 18°C, carrying a *sev-Gal4* and two *UAS-wg* transgenes, only lack interommatidial bristles and have otherwise wild-type eyes [this phenotype has also been observed by Cadigan and Nusse (1996)]. When raised at higher temperature however (29°C), this genotype results in small, bar-like, glazed eyes that are completely devoid of interommatidial bristles and show no ommatidial structures (data not shown). Consistently, no photoreceptor cells can be observed in tangential sections (data not shown). This shows that the severity of the eye phenotypes caused by ectopic *wg* expression is strongly dependent on the levels of ectopic Wg. Despite certain similarities in the phenotypes of *sev-wg* and *Gla* mutants, it appears that the time point, level, and/or position of ectopic *wg* expression in the eye imaginal disc can influence the severity and nature of the responses induced in the different ommatidial cell types.

Ectopic Expression of wg in the Developing Eye Causes Loss of spa Expression

Interestingly, *sparkling polished* (spa^{pol}) mutants, that carry a mutation in the *Drosophila* Pax-2 homolog, exhibit a characteristic eye phenotype, which resembles the *sev-wg* mutant phenotype (Figs. 5A and 5D; Fu and Noll, 1997; Brunner *et al.*, 1997). In both *sev-wg* and homozygous spa^{pol} mutants, a partial loss or misorientation of ommatidial structures and, more strikingly, an almost complete absence of the interommatidial bristles are visible in scanning electron micrographs (Figs. 5A and 5D) or in histological sections (Fig. 5B, data not shown). However, whereas in *sev-wg* transgenic animals the bristles as well as the sockets are absent (data not shown), spa^{pol} mutant animals exhibit only a loss of bristles (in spa^{pol} solely the eye-specific enhancer is deleted; Fu and Noll, 1997; Fu *et al.*,

1998). In *sev-wg/+;spa^{pol}/+* animals a strong enhancement of the *sev-wg* phenotype can be observed (data not shown). It is therefore tempting to speculate that ectopic expression of *wg* in *sev-wg* or *Gla* animals may lead to a reduction of *spa* expression in the developing eye imaginal disc. To test this hypothesis pupal eye imaginal discs were stained with an anti-Spa antibody (Materials and Methods). Indeed, Spa expression was strongly reduced or absent in bristle precursor as well as in cone cells, which normally express Spa at high levels (Fig. 5E-G; Fu and Noll, 1997), suggesting that the phenotype of *sev-wg* animals is, at least in part, a consequence of loss of *spa* expression.

In *Gla* mutant animals, ectopic *wg* expression seems to occur later in development (i.e., 24 h APF). Anti-Spa staining of pupal eye imaginal discs also revealed a consistent reduction of *spa* expression in cone cells of the central region of the discs (Fig. 5G). Surprisingly however, no reduction of *spa* was observed in bristle precursor cells. This difference in *spa* expression may explain the difference in bristle phenotypes of *Gla* and *sev-wg* mutant flies (Figs. 1B and 5A). Ectopic expression of *wg* in *sev-wg* discs occurs early enough to block the formation of interommatidial bristles [their sensory organ precursors (SOPs)] by reducing *spa* expression (see also Cadigan and Nusse, 1996; Fu and Noll, 1997). In *Gla* mutants however, ectopic *wg* may be expressed too late to interfere with *spa* expression in the bristle precursor cells and the SOPs of interommatidial bristles are formed normally.

To further test the idea that loss of *spa* expression may, at least in part, be responsible for the eye phenotypes of *sev-wg* and *Gla* mutants, we supplemented *sev-wg* animals with experimental *spa* expression. Figure 5H shows a scanning electron micrograph of a *sev-wg*/*sev-spa* individual. A clear suppression of the rough eye phenotype can be observed compared to *sev-wg* animals (Fig. 5A). To rule out the possibility that ectopic *spa* may simply act on the *sev* enhancer, *sev-spa* individuals were crossed to *sev-rough* (*sev-ro*) transformants which also have a rough eye phenotype that is dosage dependent (Basler *et al.*, 1990). In *sev-spa*/*sev-ro* animals no suppression of the *sev-ro* phenotype was observed, indicating that partial restoration of *spa* expression in *sev-wg*/*sev-spa* discs can specifically ameliorate the *sev-wg* phenotype.

DISCUSSION

The *Gla* mutation has been believed to be caused by one of the breakpoints of the inversion *In(2LR)Gla* (Morgan *et al.*, 1936). Based on the following lines of evidence however, we argue that *Gla* is a gain-of-function *wg* allele and that the *Gla* mutant phenotype results from ectopic *wg* expression caused by the insertion of a *roo* element: (i) The *Gla* mutant phenotype can be reverted by a single P element insertion into the *wg* gene of the *Gla* chromosome. (ii) A transposable *roo* element is inserted about 1 kb upstream of the *wg* transcription start site on the *Gla* chromosome. *cis*-Acting elements that are located within or near the *roo* LTR are sufficient to drive expression of a reporter gene in the developing eye tissue. The temporal and spatial activity profile of this enhancer coincides with the phenotypic aberrations that occur in *Gla* mutants during pupal eye development. (iii) Ectopic *wg* expression driven by the *sev* enhancer in the eye imaginal discs phenocopies *Gla* in various aspects.

It has previously been shown that retrotransposons contain a rich repertoire of transcriptional regulatory elements that can confer novel spatial and temporal expression patterns on the host genes into the vicinity of which they have inserted (Ding and Lipshitz, 1994). Recent studies on spontaneous mutations in various organisms have illustrated how such ectopic gene expression can cause morphological abnormalities during development and adulthood. An example is the integration of the mouse mammary tumor virus (MMTV) into the murine *wg* homolog Wnt-1 (*int-1*). The insertion of this retrovirus, which is structurally related to *Drosophila* copia transposable elements, leads to ectopic activation of the *int-1* gene in mammary glands. As a consequence, affected mice develop breast cancer (Nusse and Varmus, 1982).

We report here that in *Drosophila*, ectopic *wg* expression in the eye imaginal disc interferes with correct eye development (see also Cadigan and Nusse, 1996). Normally, during eye development Wg activity contributes to the correct spatial localization and movement of the morphogenetic furrow and specifies head cuticle at the periphery of the compound eye where no bristles are formed (Ma and Moses, 1995; Treisman and Rubin, 1995; Wiersdorff *et al.*, 1996; Royet and Finkelstein, 1996). No other functions for Wg during eye development have been described. Ectopic *wg* expression leads to the loss of photoreceptor and bristles cells, both in *sev-wg* transformants and in *Gla* mutant animals (see also Cadigan and Nusse, 1996). However, there is a difference in the severity of these phenotypes between animals of the two genotypes. Interestingly, the loss of bristles is complete in *sev-wg* transformants (carrying one copy of the transgene) but it is restricted to the anteroventral part of the *Gla* mutant eye. The penetrance however of photoreceptor cell loss is complete in *Gla* mutants, but *sev-wg* transformants lack photoreceptors only in the anteroventral part of the eye. There are two possibilities to explain this difference. A first explanation is suggested by the differing time points of ectopic *wg* expression in the two mutants. In *sev-wg* transformants *wg* is expressed immediately behind the morphogenetic furrow in the third instar disc, whereas in *Gla* mutants *roo*-driven expression starts later, i.e., 24 h after puparium formation. It is likely that both, the loss of photoreceptor and bristle cells, caused by ectopic *wg* expression, are restricted to defined time periods and that there is only a small temporal overlap in *wg* expression between *sev-wg* transformants and *Gla* mutants. Therefore, only a few photoreceptor cells are eliminated in *sev-wg* eye primordia before *wg* expression ceases. In *Gla* mutants the initial *wg* expression would just be early

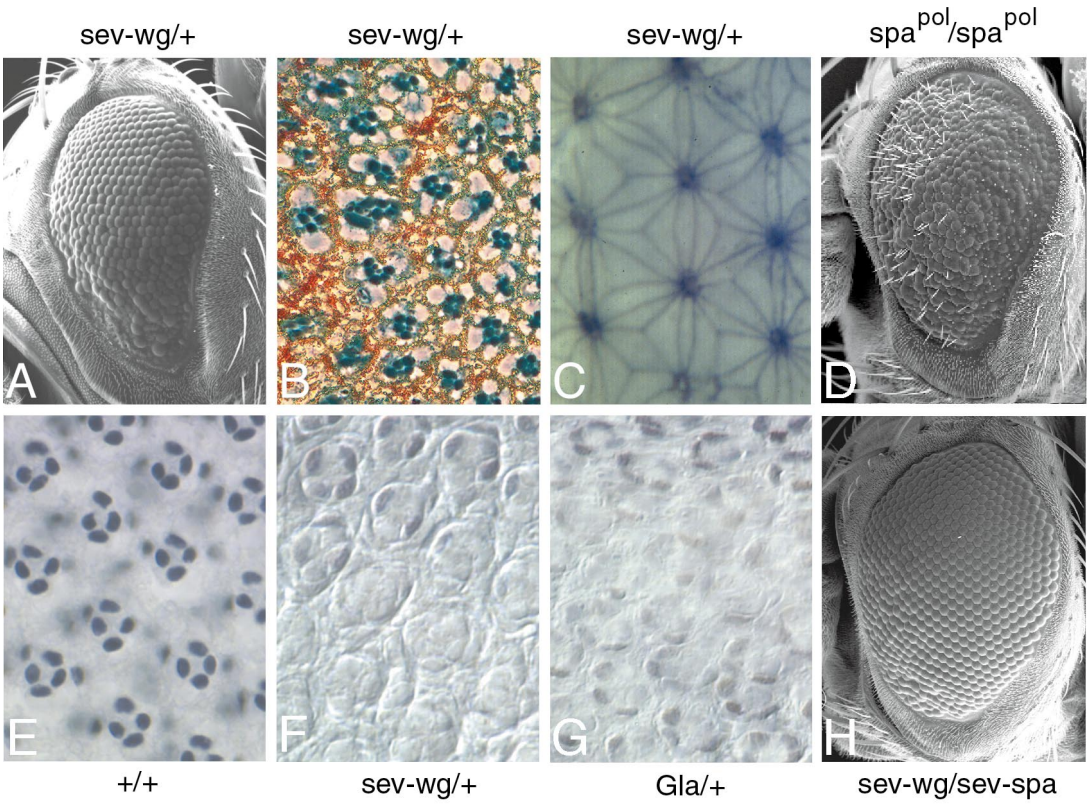

FIG. 5. Phenotypes caused by the *sev-wg* transgene. Scanning electron micrograph of a *sev-wg* eye (A). In histological sections through *sev-wg* eyes (B), misoriented and missing photoreceptor cells can be observed. Pigment granules appear to be highly abundant over the entire section. Cobalt sulfide stainings of the basal membrane of pupal eye discs (C; approximately 60 h APF) of *sev-wg* transformants show the formation of supernumerous pigment cells. Scanning electron micrograph of a spa^{pol}/spa^{pol} eye (D). Anti-Spa antibody staining of a wild-type (E), *sev-wg* (F), and *Gla* (G) pupal eye disc fixed 24 h APF. Scanning electron micrograph of a *sev-spa*/*sev-wg* eye (H). Coexpression of *sev-spa* with *sev-wg* suppresses the *sev-wg* eye phenotype (compare to A).

enough to cause at least the disappearance of some bristle cells before these become insensitive to Wg signaling (see also Cadigan and Nusse, 1996). Further investigations of the duration of *sev* enhancer activity in pupal eye discs will reveal whether a temporal overlap exists at all between *sev* and *roo* LTR-driven *wg* expression. A second explanation for the different penetrance of the two phenotypes could be found in the differing identities of the *wg*-expressing cells. In *sev-wg* eye discs photoreceptor and cone cell precursors express *wg*, whereas in *Gla* mutants *wg* is expressed by the developing pigment and bristle cells. Intriguingly, in both cases the cell types secreting Wg are those that appear least affected.

We do not completely understand why photoreceptor cells are lost from the developing eye discs expressing *wg* ectopically, but we have obtained some evidence that this might be due to the triggering of apoptosis. First, the nuclei of the disintegrating cells are fragmented like those in cells undergoing apoptosis, rather than being gradually degraded as in necrotic cells. Second, the time point of this process overlaps with the time point when normally all the undetermined surplus cells are eliminated from the developing eye disc via apoptosis (Hay *et al.*, 1994). Ectopic Wg might therefore inhibit a process that normally protects the developing photoreceptor cells from undergoing programmed cell death. These findings are strongly supported by a recent study by Ahmed *et al.* (1998) which shows that the inactivation of a *Drosophila* homolog of the tumor suppressor gene APC (D-APC) leads to increased activity of Armadillo (Arm) and Pangolin (Pan), two components required for Wg signaling (for review see Nusse, 1997; Willert and Nusse, 1998). Absence of D-APC activity causes retinal neuronal degeneration and pigment cell hypertrophy by inducing apoptotic cell death in the developing retina. The APC phenotypes are strikingly similar to those found in *sev-wg* and *Gla* animals. It is therefore tempting to speculate that the same molecular events are responsible for the phenotypes of D-APC mutants and *sev-wg* or *Gla* animals. Whether the retinal defects induced by APC loss in humans [congenital hypertrophy of the retinal pigment epithelium (CHRPE)] result from a similar sequence of events remains to be determined (Ahmed *et al.*, 1998).

We have observed that the loss of the interommatidial bristles and photoreceptor cells can be correlated with decreased or absent *spa* expression. However, we do not know whether loss of *spa* is sufficient to trigger apoptosis. In addition to the loss of photoreceptor and bristle cells, ectopic *wg* expression results in excessive eye pigmentation. Although we cannot exclude the possibility that this is due to an overproduction of pigment granules, it appears to be caused by the formation of supernumerary pigment cells: In *sev-wg* animals, additional secondary and/or tertiary pigment cells form and in *Gla* mutants secondary pigment cells appear to be transformed into tertiary pigment cells.

To understand how *wg* misexpression can induce programmed cell death and reprogram ommatidial cell fates, and how Wg functions during normal eye development, it is necessary to understand how retinal cells interpret and respond to the Wg signal. *sev-wg* transgenic and *Gla* mutant strains provide a useful tool to gain further insight into these mechanisms. Because their phenotypes are dominant and dosage sensitive, they represent a highly sensitized genetic system which can be used for genetic screens to identify new components of the Wg signaling machinery (see Cadigan and Nusse, 1996; Brunner *et al.*, 1997).

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